AWARD NUMBER:                    W81XWH-14-1-0497


TITLE:                           Preventing Cartilage Degeneration in
                                 Warfighters by Elucidating Novel Mechanisms
                                 Regulating Osteocyte-Mediated Perilacunar
                                 Bone Remodeling


PRINCIPAL INVESTIGATOR:          Tamara Alliston


CONTRACTING ORGANIZATION:        University of California San Francisco
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PREPARED FOR:   U.S. Army Medical Research and Materiel Command
                Fort Detrick, Maryland  21702-5012

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Preventing Cartilage Degeneration in Warfighters by Elucidating Novel Mechanisms Regulating Osteocyte-Mediated Perilacunar Bone Remodeling

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**6. AUTHOR(S)**

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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

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**8. PERFORMING ORGANIZATION REPORT NUMBER**

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U.S. Army Medical Research and Materiel Command
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**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT** Our overall hypothesis is that an adverse biologic response to protracted high mechanical loads compromises osteocyte-mediated perilacunar remodeling (PLR), bone quality, and cartilage health in post-traumatic osteoarthritis (PTOA). Few molecular details are known about the regulation of PLR or bone quality in healthy bone or in disease. Therefore, we will test the hypothesis that mechanical load and TGF$\beta$ signaling interact to regulate PLR, and that this regulation is impaired in, contributes to, and can be targeted for prevention of, the progression of PTOA. We are testing this hypothesis using mouse models and human PTOA tissue. We aim to determine: 1) the extent to which mechanical loading regulates PLR in a TGF$\beta$-dependent manner, 2) the relationship among PLR, strain, TGF$\beta$, and cartilage degeneration, and 3) the causality of PLR in cartilage degeneration. During the first year, we have rigorously validated approaches, developed new protocols, and generated new reagents to address these questions. The first mouse and human specimens needed to test these hypotheses have been collected and are currently being analyzed. Preliminary analysis of gene expression supports the hypothesis that PLR is TGF$\beta$-regulated. Subsequent studies will use these approaches to answer the significant questions posed by this project. Since osteocytes have not been implicated in OA, understanding their role in disease has significant potential to yield new drug targets to impede cartilage degeneration.

**15. SUBJECT TERMS**
Osteocyte, remodeling, bone, bone quality, post-traumatic osteoarthritis, TGF-beta, mechanical load, matrix metalloprotease, perilacunar remodeling, mechanobiology

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# Table of Contents

## 1. INTRODUCTION

Bone and cartilage cooperate to support healthy joint function – and both tissues are affected by, and contribute to joint disease. However, the mechanisms by which bone defects cause cartilage degeneration, and vice versa, remain unclear. To elucidate these mechanisms, this project focuses on osteocytes in subchondral bone, their ability to support joint health, and their contribution to post-traumatic osteoarthritis (PTOA). Osteocytes sense and respond to mechanical loads, and they are also known to remodel the local bone environment through a process called perilacunar remodeling (PLR). PLR is important for maintaining bone quality. We hypothesize that normally, osteocyte-mediated PLR is mechanosensitive, but that it is impaired by the excessive loads that ultimately drive PTOA. Failure of osteocyte-mediated PLR leads to bone fragility. This fragile bone may be incapable of absorbing the shock of loading that could, in turn, compromise cartilage function and ultimately lead to PTOA. This hypothesis represents an advance beyond what is known because the ability of osteocytes to remodel and strengthen bone has only recently been discovered. Consequently, the possibility of targeting osteocytes with drugs to prevent or treat PTOA has not yet been explored. Therefore, the overall objective of this project is to uncover the effect of mechanical loads on the ability of osteocytes to remodel and strengthen bone. Further, we will determine if defective remodeling causes cartilage degeneration in PTOA. We will accomplish these goals by studying human tissues collected from PTOA veterans who undergo joint replacement surgery, as well as animal models. This approach is powerful because it supports our clear goal of identifying new cellular and molecular targets that can be used therapeutically to prevent or treat PTOA.

## 2. KEYWORDS

osteocyte, remodeling, bone, bone quality, post-traumatic osteoarthritis, TGF-beta, mechanical load, matrix metalloprotease, perilacunar remodeling, mechanobiology

## 3. ACCOMPLISHMENTS

**What were the major goals of the project?**

*Major Goals*

Aim 1: Determine the extent to which mechanical loading regulates PLR in a TGF$\beta$-dependent manner.

Aim 2: Determine the extent to which PLR defects in human PTOA subchondral bone spatially correlate with strain intensity, TGF$\beta$ signaling, or articular cartilage degeneration.

Aim 3: Determine the extent of causality between defective PLR and cartilage degeneration in PTOA.

*CY14/15 Milestones and Percent Completion*

☑      IACUC Approval      –      100% complete

☑      CHR Approval      –      100% complete

**What was accomplished under these goals?**

**Aim 1: Determine the extent to which mechanical loading regulates PLR in a TGFβ-dependent manner.**

*Major Task 1: Determine the mechanosensitivity of PLR and PLR proteins using loaded SBE-Luc mice*

<u>Subtask 1:</u> *Examine mechanosensitivity of PLR using histology, second harmonic generation microscopy (SHG), and microCT*

<u>Subtask 2:</u> *Examine the mechanosensitivity of proteins implicated in PLR using immunohistochemistry (IHC)*

<u>Subtask 3:</u> *Examine the mechanosensitivity of TGFβ activity in subchondral bone in areas of PLR using IHC*

We have made significant progress on Major Task 1, though the results of these experiments are still pending. First, we expanded the breeding colony of SBE-Luc mice to facilitate these studies. Second, we recalibrated our mechanical loading apparatus and the strains applied to the SBE-Luc hindlimb using strain gauge testing. Third, based on developments from other laboratories since the submission of this proposal, we decided to test key parameters in our loading regimen to determine which would be most appropriate for this study. Bones used in this analysis are being processed histologically for Subtasks 1-3.

More specifically, in this experiment we compared mouse age (8 vs. 12 weeks), frequency (1 vs. 2 Hz), and load (3.5 vs. 5 N). A total of twelve SBE-Luc mice, distributed among these conditions (N=3/condition), received 10 min of unilateral hindlimb loading for 5 sequential days. To identify the best loading condition, we evaluated the effect of each condition on bone mass (micro-CT analysis of cortical BV/TV) and TGFβ signaling (Western analysis of phospho-Smad3 levels). Though all of these conditions produced the anticipated increase in cortical bone volume, the 8-week, 3.5N, 1Hz condition produced the most consistent and greatest magnitude differences in cortical bone and TGFβ activity **(Figure 1)**. Specifically, these conditions produce a significant 13% increase in cortical bone volume (p<0.05). Bones from this study are undergoing histologic processing for the evaluation of PLR parameters and TGFβ signaling as proposed in Subtasks 1, 2, and 3. Though a definitive answer about the mechanosensitivity of PLR will likely not be in hand as projected for the 13 month Milestone, we will have preliminary data by that time, and are on track to answer this question within Year 2.
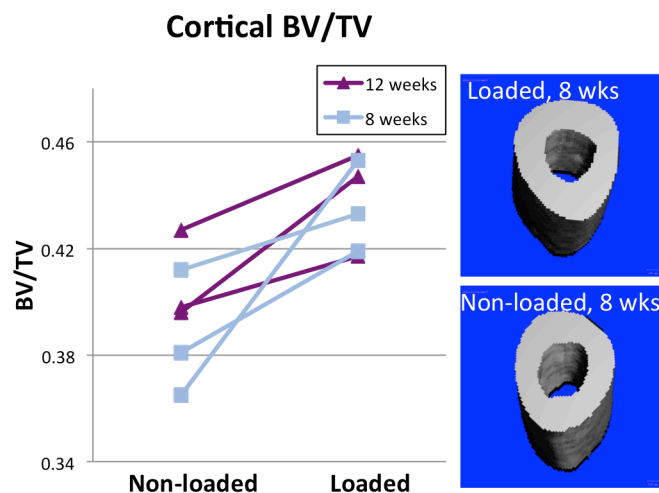


*Figure 1: In vivo loading model induces bone apposition differentially with mouse age.* The left panel shows an average 10% increase in bone mass for all mice loaded at 3.5 N, 1 Hz for five days. The difference in 8-week old mice (13%) is significant, while the difference for 12-week old mice (8%) is not significant. Micro-CT scans show a difference in cortical thickness.

***Major Task 2:*** *Determine if TGFβ regulates PLR and is required for its mechanosensitivity using loaded SBE-Luc mice treated with SD208 or vehicle*

<u>*Subtask 1:*</u>  *Examine mechanosensitivity of PLR using histology, SHG, and microCT*

<u>*Subtask 2:*</u>  *Examine the mechanosensitivity of proteins implicated in PLR using IHC*

To evaluate the effect of TGFβ on PLR, we treated 8-week old mice (N=8/group) with vehicle or the TβRI-inhibitor SD-208 (60 mg/kg) for 6 weeks.  This is a critical step toward this goal of evaluating the role of TGFβ in the mechanosensitivity of PLR (the results of which are still pending, as described in Major Task 1 above).  We verified the previously reported increase in BV/TV using micro-CT to confirm the efficacy of the SD-208 treatment (p<0.05).  Bones from this experiment were isolated for analysis in Subtasks 1-2.

Although analysis of all of the histological outcomes is still underway, our preliminary analyses of PLR gene expression shows that inhibition of TGFβ signaling with SD-208 represses the expression of several key PLR enzymes, including MMP13 (40% repression, p=0.06), MMP14 (60% repression, p<0.05), and Cathepsin K (70% repression, p<0.05). Genes implicated in PLR that did not change in response to SD-208 include SOST, carbonic anhydrase 2,TIMP2, and MMP2.   The extent to which these changes in gene expression are due to osteocyte-intrinsic differences remains to be determined using immunohistochemcial analyses.  Current data, though preliminary, lend additional support to our hypothesis that TGFβ participates in the regulation of PLR.  This part of the project is currently ahead of schedule (12-24 mos), but will likely require all of Year 2 to definitively answer, as projected.

***Major Task 3:*** *Determine the effect of load and TβRI-I on perilacunar mineralization and material properties using loaded SBE-Luc mice treated with SD208 or vehicle*

<u>*Subtask 1:*</u>  *Evaluate bone matrix mineralization using microCT*
Bones from TβRI-I treated mice have been assessed using X-ray tomographic microscopy to evaluate matrix mineralization and lacunar size.   Though the measurements are complete, the results are yet unknown because reconstruction of these complex datasets is computationally-intensive and time-consuming. This analysis is ahead of schedule (20-24 mos).

<u>*Subtask 2:*</u>  *Examine bone matrix material properties using scanning probe microscopy*
A new scanning probe microscopy module will be installed on the atomic force microscope (AFM) to be used for these studies in the spring of 2016.  This user-friendly upgrade will simplify the operation of the AFM and the measurements as well as the quantitative analyses.  Our team will be trained on the new module in during Year 2 in preparation for these analyses (scheduled for 20-24 mos).  Bones from SD-208 and vehicle-treated mice have been collected for this analysis.

**Aim 2.  Determine the extent to which PLR defects in human PTOA subchondral bone spatially correlate with strain intensity, TGFβ signaling, or articular cartilage degeneration.**

Though we have actively been working on Aim 2, this Aim is behind schedule.   We originally anticipated that the proposed use of surgical waste specimens was exempt. However, these studies require CHR approval, which resulted in a significant delay.  CHR

approval was granted, from UCSF, SFVAMC, and DOD, in June 2015, marking the completion of an important Milestone in our project.

Throughout Year 1, our research team (including the clinician scientists), has been meeting monthly, both to complete a similar analysis of osteonecrotic human femoral heads from an independent project and to initiate the studies proposed in Aim 2. Recognizing the vast differences in the size, shape, and condition of the tibial plateau specimens from the femoral heads, our team decided to complete the analysis of one tibial plateau prior to collecting additional specimens *(Figure 2)*. In this way, we are validating all of the collection, processing, and analysis protocols. Currently, the samples from this pilot specimen are undergoing histological processing, which takes approximately 1 month due to decalcification. We anticipate completion of this pilot analysis in the first quarter of Year 2, after which we intend to proceed with the collection and analysis of additional specimens from PTOA patients and from cadaveric donors. We anticipate that we can move more rapidly in Year 2 as the CHR approval is in place and our protocols for collecting and analyzing the human tibial plateau tissues are now being established.
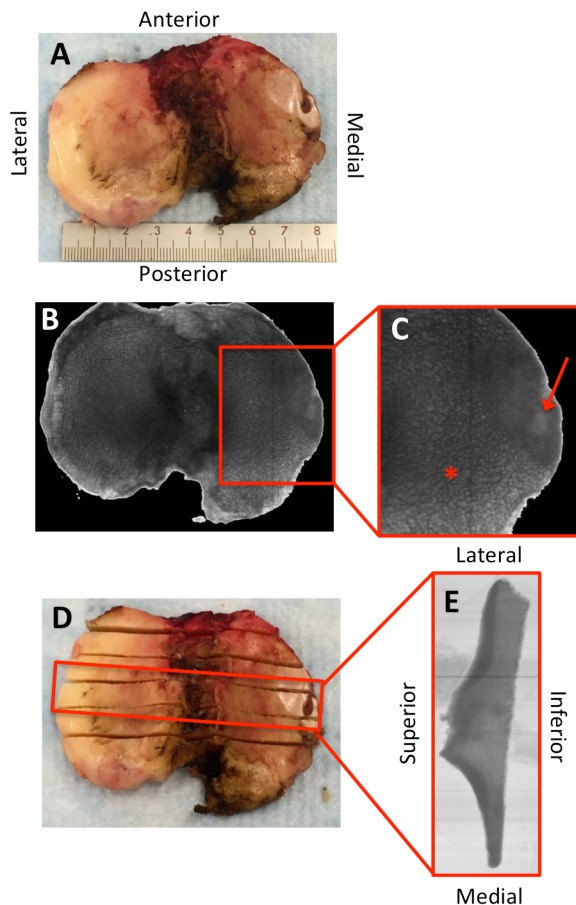


*Figure 2. Tibial plateaus from patients with post-traumatic osteoarthritis are collected as surgical waste tissue following knee arthroplasty for radiographic and histological analyses.* Samples are photographed and measured for documentation of gross phenotype and orientation (A), prior to radiographic analysis with plain X-ray (B) to identify areas of less affected bone (C, red asterisk) and bony lesions (C, red arrow). Based on this analysis, specimens are cut into 8 mm wide slabs in the lateral to medial direction (D). Each slab is X-rayed for documentation (E) prior to fixation and decalcification for histology, or storage for analysis of bone quality.

**Aim 3. Determine the extent of causality between defective PLR and cartilage degeneration in PTOA.**

*Major Task: Determine if PTOA causes PLR defects and if PLR defects cause PTOA*

*Subtask 1:* Breed mice

Aim 3 requires DMP1-Cre;MMP13$^{fl/fl}$ mice.  We secured the DMP1-Cre mice from Dr. Lynda Bonewald and the MMP13$^{fl/fl}$ mice commercially.  We bred the two strains.  After multiple generations, breeding pairs of the needed genotypes have been established.  The first cohort of mice of the desired genotypes have recently reached 8 weeks of age, at which point, the bones were harvested for initial characterization of the basal MMP13$^{OCY-/-}$ phenotype.  This task, originally scheduled for Year 2 (12-24 mos) is significantly ahead of schedule.

*Subtask 2:  Perform MCI surgeries*
Once the basal phenotype of the MMP13$^{OCY-/-}$ mice is characterized and sufficient numbers of mice are available, we will proceed with the MCI studies proposed in Subtask 2. Meanwhile, we have ordered the supplies needed for the surgical procedures and procured space in the animal facility to perform this survival surgery.  In addition, Dr. Fowler has learned the procedure from Dr. Dang.  Therefore, when the mice are available, these studies will rapidly progress, which is ahead of the original schedule (24-28 mos).

*Subtask 3:  Histologic analysis of PLR and cartilage (SHG, Mankin scoring, IHC)*
Characterization of the basal MMP13$^{OCY-/-}$ phenotype is required to interpret the PTOA phenotype in the MCI model, as described in Subtask 3.  For the evaluation of the basal phenotype, we have so far collected bones from 3 male and 3 female 2-month old "WT" (DMP1-Cre$^{-/-}$; MMP13$^{fl/fl}$) and 3 male and 3 female 2-month old MMP13$^{OCY-/-}$ (DMP1-Cre$^{+/-}$; MMP13$^{fl/fl}$) mice.

As described in Subtask 3, MMP13$^{OCY-/-}$ bones are undergoing micro-computed tomography and histologic analysis, as well as analysis of expression of genes implicated in PLR.  All of these analyses are currently underway.  This analysis of the basal phenotype lays a strong foundation for the evaluation of the PTOA phenotype.  This activity, proposed for Year 3 (26-36 mos) is significantly ahead of schedule.

**What opportunities for training and professional development has the project provided?**

The primary training opportunity has been for Dr. Tristan Fowler, who has learned several new approaches including histologic analyses of PLR, as well as the handling and analysis of human surgical samples.  He also has learned the MCI technique from Dr. Dang.  Over the coming year, he will prepare abstracts for national meetings and manuscripts for publication.  Throughout the course of the first year, Dr. Fowler has mentored rotating graduate, undergraduate, and medical students, as well as residents in orthopaedic surgery.  This has provided a professional development opportunity for Dr. Fowler, as well as for the students he has mentored.

**How were the results disseminated to communities of interest?**

Given the early stage of the project, we have not yet disseminated the results.  We intend to submit abstracts to the ASBMR and ORS meetings in the coming year.

**What do you plan to do during the next reporting period to accomplish the goals?**

The primary goal of Year 2 is to determine the extent to which PLR is mechanosensitive. We have several major milestones:

☐ Establish the mechanosensitivity of PLR

☐ Establish TGFβ-dependence of PLR
☐ Establish the mechanosensitivity of bone quality
☐ Establish the integrity of PLR in PTOA bone
☐ Determine if PTOA uncouples loading, TGFβ, and PLR
☐ Establish whether cartilage degeneration occurs in areas of disrupted PLR

To accomplish these milestones, we will continue with the ongoing histologic and molecular studies of PLR mechanosensitivity and TGFβ-sensitivity as proposed in Aim 1. In addition, we will prepare for and undertake the corresponding analyses of bone quality. We will continue to collect and process the human cadaveric and surgical specimens for Aim 2, including the finite element analyses. For Aim 3, following the characterization of the basal MMP13$^{OCY-/-}$ mouse phenotype, we will proceed with the MCI injury studies. In addition, we intend to prepare a manuscript detailing a set of validated PLR protocols for submission to a peer-reviewed publication such as *JBMR* or *Bone*.

## 4. IMPACT

**What was the impact on the development of the principal discipline(s) of the project?**

The greatest impact of our work on this project at the completion of the first year is the development and validation of rigorous and quality controlled methods for these studies. Several of the approaches we use require pushing the boundaries of existing methods to answer new questions. One specific way that this approach impacts the field is by developing methods that significantly improve the ability to rigorously evaluate PLR. For example, previous methods allow visualization, but not quantification, of canalicular density on paraffin histological sections. We had tried numerous currently available protocols, with great variability in staining efficiency, particularly across bone types or between mouse and human bone. We have since developed a highly reproducible and quantitative approach that can be applied to paraffin sections. This is important because canalicular density can then be analyzed in the same bone, and in adjacent sections to, those used for immunohistochemical analysis of PLR enzymes or collagen organization. We are also developing approaches to use X-ray tomographic microscopy to quantify and visualize lacunar size in 3D instead of 2D sections. Given that PLR is spatially controlled within bone, we are confident that these approaches will advance the field technically as well as scientifically. We are preparing a manuscript on PLR methods to share these protocols with our community.

Most importantly, these advances provide us with a solid foundation to answer the scientific questions posed by this project within Year 2. The impact of understanding the mechanoregulation of PLR, as well as understanding the potential to regulate bone quality, is of pivotal importance, both for understanding the control of normal bone as well as the mechanisms that are deregulated in post-traumatic osteoarthritis.

**What was the impact on other disciplines?**                    None

**What was the impact on technology transfer?**                  None

**What was the impact on society beyond science and technology?**   None

## 5. CHANGES/PROBLEMS

**Changes in approach and reasons for change**

Because of the delay in Aim 2 (described below), we pursued other aspects of the project on which we could make progress sooner than projected in our original timeline. We have not changed our goals, only accelerated parts of Aims 1 and 3 that were originally scheduled to begin in Year 2.

**Actual or anticipated problems or delays and actions or plans to resolve them**

Our study encountered two unanticipated but significant delays in Year 1. The Staff Research Assistant unexpectedly left this position in January 2015. We recruited a new Staff Research Assistant in July 2015. He has been assisting Dr. Fowler with the management of the mouse breeding and genotyping.

Although we anticipated in our DOD proposal that this research would be classified as 'Exempt', it required CHR Review and Approval. This significantly delayed Aim 2 because of the need to secure human subjects approval from UCSF, SFVAMC, and DOD. This process was completed near the end of the 3rd quarter of the project, in July of 2015. Aim 2 studies are now underway. Therefore, each of these issues has been resolved, and our progress is much more rapid with these changes in place.

**Changes that had a significant impact on expenditures**

Year 1 was under budget due to the unexpected departure of a staff member. We will carry these funds forward into Year 2 to pay for the additional personnel effort that will be needed to complete the proposed studies. This will equalize the expenditures from the Year 1 budget.

Though we have not yet performed the studies, the increased number of human specimens to be analyzed (12 instead of 8) will require increased expenditures above what was budgeted in Year 2 of the project.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Based on sample variability in an independent but similar study, we requested an increase from the originally proposed 8 surgical samples to 12 samples, which was approved by the UCSF, VA, and DOD CHRs. No changes were made in the care or use of vertebrate animals, biohazards, and/or select agents


## 6. PRODUCTS

**Publications, conference papers, and presentations:**

Though this project is in its early stage, the PI was invited to <u>moderate a session</u> and <u>speak</u> on the topic of osteocyte-perilacunar remodeling at the *ORS Sun Valley Mineralized Tissue Workshop* in Sun Valley, ID on August 5, 2015. Dr. Alliston invited speakers to discuss the topic, presented an introduction that reviewed the state of current knowledge and outstanding questions on the topic of PLR, and also presented a research talk entitled "Osteocyte-mediated control of bone quality through perilacunar remodeling." Although the

data presented derived from other projects, these efforts are products of this DOD-supported project because the knowledge base developed in Year 1 advanced our ability to answer those questions and to share these concepts with a larger group of investigators in bone biology.

**Website(s) or other Internet site(s):**                                    None

**Technologies or techniques:**

We have developed and validated an improved methodology for the analysis of perilacunar remodeling. Given the importance of robust methods to quantitatively evaluate perilacunar remodeling for the entire project, we have invested a significant amount of effort in improving and validating these outcomes on murine and human bone samples leftover from unrelated studies. Specifically, we have improved the protocols for the analysis of lacunocanalicular area by silver staining and for analysis of collagen alignment by polarized light microscopy of picrosirius red staining. Final protocol validation for these two approaches is complete. We continue to work on the optimization of other PLR outcomes and intend to write a manuscript of PLR methods in the coming year.

**Inventions, patent applications, and/or licenses:**              None

**Other Products:**                                                          None


## 7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

**What individuals have worked on the project?**

Only individuals who have contributed more than 1 person month per year are listed.

| Name: | Tamara Alliston |
|---|---|
| Project Role: | PI |
| Nearest Person Month Worked: | 1 |
| Contribution to Project: | Lead the project. |
| Funding Support: | DOD, NIH, UCSF |

| Name: | Tristan Fowler |
|---|---|
| Project Role: | Post-doctoral Scientist |
| Nearest Person Month Worked: | 8 |
| Contribution to Project: | Perform experiments. |
| Funding Support: | DOD, NIH |

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

*Tamara Alliston*
Although the PI's effort on the DOD project (1.2 mos) has not changed, the PI's role on the following projects has concluded due to the end of the grants:
   NIH R01: Mesenchymal Regulation of Osteogenesis (0.6 mos)

   NIH R21: Structured co-culture of stem cells and chondrocytes for spinal disc repair (1.2 mos)

Orthofix:  Effects of Dosing and Magnetic Micro-rods on the Intervertebral Disc Cells (As Needed)

Hearing Research Inc:  Sclerostin-mediated protection of cochlear bone remodeling (As Needed)

UCSF - Bridge Funding for Skeletal Biology Research (As Needed)

The PI has new roles due to the award of these grants, none of which overlaps with the current project:

NIH R21:  The Mechanobiology of TGF-beta Signaling in Chondrocytes (1.8 mos)

NIH P30:  Core Center for Musculoskeletal Biology and Medicine (0.6 mos)

Hearing Research Inc: MMP-13-mediated cochlear bone remodeling (As Needed)

NIH R01:  The mechanistic control of bone extracellular matrix material properties by TGF$\beta$ (3.6 mos)

UC/MEXUS:  Role of Osteocytes in the Development of Bone Metastases (As Needed)

*Jeffrey Lotz*
Dr. Lotz's role on the following projects has concluded due to the end of the grants:

NIH R21:  Structured co-culture of stem cells and chondrocytes for spinal disc repair

Orthofix:  Effects of Dosing and Magnetic Micro-rods on the Intervertebral Disc Cells

Dr. Lotz has a new role due to the award of this grant, which does not overlap with the current project:

NIH R34:  University of California Tissue Regeneration Resource Center (UCTRRC) (0.6 mos)

*Thomas Vail*
No Changes.

*Alfred Kuo*
No Changes.

*Alexis Dang*
Dr. Dang has a new role due to the award of this grant, which does not overlap with the current project:
VA SPIRE:  Role of Progranulin in endochondral ossification after spinal cord injury (2 mos)

**What other organizations were involved as partners?**

*Organization Name:*        San Francisco VA Medical Center

*Location of Organization:*        4150 Clement Street

*Partner's Contribution:*
    Facilities:        Surgical specimens are collected at the SFVAMC Hospital
    Collaboration:        SFVAMC staff, Alfred Kuo and Alexis Dang, are collaborators.

**8.  SPECIAL REPORTING REQUIREMENTS**

**Collaborative Awards:**     Not Applicable

**Quad Charts:**                    Please see Appendix 1.


**9.  APPEDICES**

**Appendix 1:**                     Quad Chart for Year 1

# Preventing Cartilage Degeneration in Warfighters by Elucidating Novel Mechanisms Regulating Osteocyte-Mediated Perilacunar Bone Remodeling (PLR)

OR130191

**PI:** Alliston **Org:** UCSF **Award Amount:** $500,000

## Study Aims

- Determine the extent to which mechanical loading regulates PLR in a TGFβ-dependent manner.

- Determine the extent to which PLR defects in human PTOA subchondral bone spatially correlate with strain intensity, TGFβ signaling, or articular cartilage degeneration.

- Determine the extent of causality between defective PLR and cartilage degeneration in PTOA.

## Approach

We hypothesize that mechanical load and TGFβ regulate PLR, and further, this regulation is impaired in and contributes to PTOA. To test this hypothesis, perilacunar bone remodeling (PLR) will be examined in genetically modified mice following one-limb loading, TGFβ-inhibition, or traumatic joint injury. To relate these findings to human PTOA, PLR will also be evaluated in human knee specimens recovered following total joint replacement surgery. The effect of defective PLR on cartilage will be evaluated in human and murine specimens.
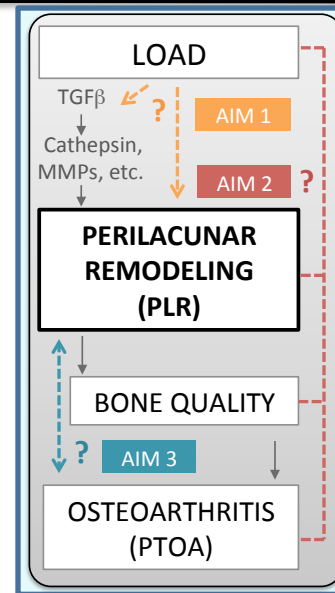


Accomplishments:

1. Milestone Completion: IACUC and ACURO approval secured, protocol OR130191, Date: 28-02-2015

2. Milestone Completion: UCSF and DOD approval for the use of cadaveric tissues, protocol A-18336.1, Date: 25-03-2015

3. Milestone Completion: The study is not exempt from human subjects review. Thus, human subjects approval has been secured from SFVAMC (02-04-2015), UCSF (09-05-2015), and DOD (11-06-2015).

## Timeline and Cost

| Activities CY | 9/14–8/15 | 9/15-8/16 | 9/16-8/17 |
|---|---|---|---|
| Determine the mechanosensitivity of PLR and PLR proteins | ■ | | |
| Determine if TGFβ regulates PLR and is required for its mechanosensitivity | | ■ | |
| Determine the effect of load and TβRI-I on perilacunar mineral and material properties | | | ■ |
| Determine if regulation of PLR and TGFβ in human subchondral bone is lost in PTOA | ■ | | |
| Determine the effect of PLR on bone quality in PTOA | | | ■ |
| Determine if PTOA causes PLR defects and if PLR defects cause PTOA | | ■ | |
| **Estimated Budget** ($500,000 direct) | **$168,151** | **$190,281** | **$141,568** |

**Updated: 10/30/15**

**Milestones**

**CY14/15 Milestones** – studies approved and underway
- ☑ Local IACUC Approval
- ☑ CHR Exempt status approved

**CY15/16 Milestones** – investigate mechanisms regulating PLR
- ☐ Establish mechanosensitivity of PLR
- ☐ Establish TGFβ-dependence of PLR
- ☐ Establish the mechanosenstivity of bone quality
- ☐ Establish the integrity of PLR in PTOA bone
- ☐ Determine if PTOA uncouples loading, TGFβ, and PLR
- ☐ Establish whether cartilage degeneration occurs in areas of disrupted PLR

**CY16/17 Milestones** – determine the causality of PLR and PTOA
- ☐ Establish the extent to which PTOA disrupts PLR
- ☐ Establish the extent to which PLR defects cause PTOA

**Comments/Challenges/Issues/Concerns**
We will change the bars on the timeline to reflect revised order of studies.

**Budget Expenditure to Date**
Projected Expenditure: $168,151
Actual Expenditure: $ 113,484